# Studies on the Pectic Enzymes
## I. Action of Pectinase

By

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## I. Introduction

Protopectinase is the enzyme that dissolves the middle lamella. Since the work of DAVISON and WILLAMAN, it has been considered that protopectinase and pectinase are two distinct enzymes. They presented evidence that two enzymes are different in their occurrence, temperature of heat inactivation, pH optimum and their behavior during adsorption and precipitation. But protopectinase is a hypothetical enzyme. Because the chemical structure of protopectin is still uncertain. COLIN and CHAUDUN hold the view that there is no preliminary breaking up under the action of hypothetical protopectinase when the maceration of plant tissues is caused by the enzyme.

The discovery of HENGLEIN and SCHNEIDER that pectic substances have a high molecular cellulose-like structure, started a new era in pectin chemistry. From the new standpoint, a systematic study was undertaken at this laboratory to determine whether protopectinase is identical with pectinase. The results obtained are presented here.

## II. Experimental Part

1. Preparation of Substrate. The dried marc of Satsuma orange was extracted with hot water and pectin was precipitated by adding three volumes of 95% alcohol to the filtrate. From the pectin obtained, pectic acids (tetra-galacturonic acid a and c) were prepared by the method of EHRICH. The pectin, containing a large amount of araban, was prepared from the peel of sour orange according to EHRICH. The results on the composition of substrates are shown in Table 1.

**Table 1. Composition of Substrates**

| Substrate | Ash % | Anhydro-galacturonic acid % | $CH_3O$ % | Araban % |
|---|---|---|---|---|
| Tetra-galacturonic acid a | 3.45 | 89.98 | 2.05 | 3.47 |
| Tetra-galacturonic acid c | 0.77 | 90.56 | — | — |
| Pectin containg a large amount of araban | 5.16 | 28.84 | 10.70 | 24.74 |

2. Preparation of Crude Enzymic Extracts. *Penicillium expansum* and *Rhizopus tritici* were grown on the malt juice in flasks at 27°C. The mycelia were harvested, washed in water and dried over calcium chloride in a desiccator. The dried mycelia were ground and the extracts from these powders were obtained by digesting them in water (in the proportion of 1.0gr. to 20CC. of water) at 35°C for 20 hours.

3. Action of Pectinase. Tetra-galacturonic acid a and pectic acid containing a large amount of araban were dissolved in a required volume of $^{N}/_{10}$ NaOH solution respectively, saponified by standing for 2 hours and neutralized with HCl. Each reaction mixture, total volume 100cc., contained substrate 1.0gr., crude enzymic extract 10cc. and McIlvaine's buffer 15cc to adjust the solution to pH 5.0. The mixtures were kept at 35°C. The WILLSTÄTTER-SCHUDEL method was used for the determination of the increase in the reducing powder. The viscosity determinations were performed at 25°C, using OSTWALD viscosity pipets. The results obtained on the changes in the viscocity and reducing power of reaction mixtures are expressed as in Table II and Table III.

**Table 2. Changes in the Reducing Power and Viscosity of Tetra-galacturonic Acid a Solution**

| Reaction time (hr.) | R. tritici | | P. expansum | |
|---|---|---|---|---|
| | N/50-$I_2$ cc. | Rel. visc. | N/50-$I_2$ | Rel. visc. |
| 0 | 0 | 1.230 | 0 | 1.288 |
| 3.5 | 0.54 | 1.054 | 0.16 | 1.220 |
| 6 | 0.69 | 1.050 | 6.56 | 1.176 |
| 22 | 2.64 | 1.042 | 2.47 | 1.082 |
| 27.5 | 2.60 | 1.036 | 3.20 | 1.067 |
| 43.5 | 3.19 | 1.036 | 4.35 | — |

**Table 3. Changes in ihe Reducing Power and Viscosity of Pectin Containing a Large Amount of Araban**

| Reaction time (hr.) | R. tritici | | P. expansum | |
|---|---|---|---|---|
| | N/50-$I_2$ cc. | Rel. visc. | N/50-$I_2$ cc. | Rel. visc. |
| 0 | 0 | 1.118 | 0 | 1.179 |
| 3.5 | 0.77 | 1.098 | 0.79 | 1.158 |
| 5.5 | 0.99 | 1.080 | 1.14 | 1.149 |
| 10.0 | 1.02 | 1.081 | 1.38 | 1.126 |
| 19.0 | 1.01 | 1.089 | 1.56 | 1.118 |
| 25.5 | 1.29 | 1.079 | 1.88 | 1.107 |
| 42.0 | — | 1.091 | 1.88 | 1.115 |

The data were corrected with blanks.

From the results given in Table II and Table III, it is seen that when the pectin solutions were exposed to the action of enzymic extract of *R. tritici*, decreases in the viscosity were rapid and increares in the reducing power were slow. By the action of extract of *P. expansum*, changes in the viscosity and reducing power were

both gradual. After 22 hours reation, increases in the reducing power became more rapid by the extract of *P. expansum* than by that of *R. tritici.*

4. Fractional Alcoholic Precipitation of Enzymes. Fractional precipition was tried on the extract of dried micelium of *P. expansum,* The method adopted was to add alcohol up to 50% by weight, filter precipitate I after 30 minutes of standing at 0°C, increase the concentration of alcohol up to 75% and filter another precipitate II after standing for 30 minutes. The precipitates were dissolved in water in the proportion of 5.0cc of water to 1.0g of dried mycelium. Table IV gives the results.

**Table 4. Action of Enzyme Solutions Fractionated by Alcohol**

| Enzyme | 5hr. | | 15hr. | | Rate of protopectinase activity |
|---|---|---|---|---|---|
| | N/50-$I_2$ cc. | Decrease in rel. visc. | N/50-$I_2$ cc. | Decrease in rel. visc. | |
| I | 0.47 | 0.173 | 1.07 | 0.199 | ± |
| II | 0.32 | 0.515 | 0.92 | 0.611 | ++ |

The reaction mixture, total volume 20cc, contained tetra-galacturonic acid a 0.2gr, enzyme I or II 3.0cc. and McIlvaine's buffer 3.0cc., adjusting the pH of the mixture to 4.2. The methods for determination of reducing power and viscosity were as described in experiment 3. From the results obtained, it will be seen that protopectinase and pectinase activities measured by the changes in viscosity, increase with the percentage of alcohol used. At the same time pectinase activity determined by the production of reducing sugars decreased.

5. Isolation of a Intermediate Degradation Product of Tetra galacturonic acid c. The enzymic hydrolysis was carried out by dissolving 4.0gr. of tetra-galacturonic acid c in 200cc of dilute NaOH solution adding 100cc of enzymic extract of *R. tritici* and diluting with water to 400cc. After keeping 68 hours at 35°C, 5.0cc of 4N $H_2SO_4$ and 555cc. of 95% Alcohol were added in the reaction mixture. The solution was then filtered and alcohol added until the alcoholic strength of the solution is 75%. The precipitate was washed in 85% alcohol until the wash liquid was free from SO". After washing in absolute alcohol and ether, the precipitate was dried at 50°C.

It contained 18.96% of ash, 64.86 of uronic acid and reduced Fehling's solution 40.4% as strong as d-galacturonic acid.

6. Enzymic Hydrolysis of the Intermediate Degradation Product of tetra-galacturonic acid c. 10cc. of 2% intermediate degradation product of tetra-galacturonic acid c,

**Teble 5. Enzymic Hydrolisis of Tetra-galacturonic Acid a and Intermediate Degradation Product of Tetra- galacturonic Acid c.**

| Substrate | Enzyme | 5hr. N/50-$I_2$ cc. | 13hr. N/50-$I_2$ cc. |
|---|---|---|---|
| Tetra-galacturonic acid a | I | 0.32 | 0.70 |
| | II | 0.38 | 0.83 |
| Intermediate degradation product | I | 0.38 | 0.83 |
| | II | 0.12 | 0.24 |

and 1,2cc of enzyme I or 3.0cc of enzyme II were mixed and water was added to a total volume of 20cc. The reaction mixtures were adjusted to pH 4.2 and kept at 35°C. At definite intervals a 2cc sample was withdrown from the reaction mixture and titrated. The results are given in Table V.

According to the results given in Table V, the intermediate degradation product of tetra galacturonic acid c was hydrolyzed more rapidly by the action of enzyme I than enzyme II, while tetra galacturonic acid c are decomposed almost similarly by the enzyme I and II.

## III. Discussion.

On the basis of the results expressed in Table II and IV, it may be proposed that there are two enzymes for the cleavage of glycoside linkage in pectin molecule, namely, viscosity-decreasing pectinase and sugar-producing pectinase. The activity of protopectinase seems to parallel the viscosity-decreasing activity in pectin solution. From the results given in Table V, there seems to be oligase to hybrolyze the oligosaccharide produced from pectin. These results may lead to the theory that polygalacturonic acid is first converted by the action of polyase into digalacturocic acid, the latter then hydrolyzed to d-galacturonic acid by oligase and the maceration of plant tissues caused by polyase.